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Heterospecific aggression bias towards a rarer colour morph

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Colour polymorphisms are a striking example of phenotypic diversity, yet the sources of selection that allow different morphs to persist within populations remain poorly understood. In particular, despite the importance of aggression in mediating social dominance, few studies have considered how heterospecific aggression might contribute to the maintenance or divergence of different colour morphs. To redress this gap, we carried out a field-based study in a Nicaraguan crater lake to investigate patterns of heterospecific aggression directed by the cichlid fish, *Hypsophrys nicaraguensis*, towards colour polymorphic cichlids in the genus *Amphilophus*. We found that *H. nicaraguensis* was the most frequent territorial neighbour of the colour polymorphic *A. sagittae*. Furthermore, when manipulating territorial intrusions using models, *H. nicaraguensis* were more aggressive towards the gold than dark colour morph of the sympatric *Amphilophus* species, including *A. sagittae*. Such a pattern of heterospecific aggression should be costly to the gold colour morph, potentially accounting for its lower than expected frequency and, more generally, highlighting the importance of considering heterospecific aggression in the context of morph frequencies and coexistence in the wild.

1. Introduction

A major endeavour of evolutionary ecology is to understand the processes that underlie the remarkable diversity that exists both within and between species. One of the most striking examples of this diversity is seen in polymorphic species that exhibit consistent genetic variation in coloration within populations [1–3]. Indeed, studies of such species have provided evolutionary biologists with important insights into mechanisms underpinning individual variation and phenotypic diversity, as well as mechanisms of speciation [2–6]. Yet, despite these insights, the actual sources of selection that allow the persistence of different morphs within the same population remain poorly understood [3].

In the absence of specific selection pressures that permit the coexistence of different morphs, a better performing morph would soon be expected to drive others from the population. Even in the absence of any performance advantages, one might expect the type of variation represented by discrete morphs to simply erode from the population through genetic drift and other random events [7,8]. However, morph coexistence can be favoured if a rarer morph has an advantage that is tempered at higher frequencies [9–13].

One mechanism that can act as a powerful selective force affecting the evolution of colour polymorphisms and morph frequencies is aggressive interactions. For example, individuals of a particular colour morph may enjoy fitness advantages from being more aggressive than others [14,15]. In Gouldian finches (*Erythrura gouldiae*), for instance, red-headed birds, which naturally occur at lower frequencies in the wild, aggressively dominate the more common black-headed individuals. This advantage, however, is counterbalanced at higher frequencies by stress costs of frequent aggressive interactions [16,17]. Yet, despite the apparent importance of aggression, few studies have considered its role in contributing to the maintenance or divergence of different phenotypes.

Among the few notable exceptions, the focus has almost exclusively been on patterns of aggression between the different phenotypes or morphs [18,19]. However, it is important to note that competitive biases may have evolutionary consequences not only within species, but also in the context of heterospecific interactions. For example, in sympatric species of *Hetaerina* damselflies, heterospecific intruders elicited less aggression than conspecific intruders in species pairs with dissimilar wing coloration. By contrast, in species pairs where wing coloration was more similar, heterospecific intruders were attacked as aggressively as conspecifics [20]. Such biased aggressive encounters can have important ecological and evolutionary consequences by affecting patterns of species distribution and coexistence [21]. Indeed, aggressive interactions between species are actually very common, and can be as intense as aggression within a species [22,23]. Nevertheless, the evolutionary consequences of heterospecific aggression have often been overlooked [22–24].

The crater lakes of Central America provide an excellent experimental setting for investigating the role of heterospecific aggression on patterns of coexistence and relative colour morph frequencies. Among the species of cichlid fish that coexist in these lakes, the most prominent are members of the Midas cichlid complex *Amphilophus* spp. (*sensu* [25–27]). Many species within this group display discrete, but naturally co-occurring, and genetically inherited 'dark' and so called 'gold' (typically orange in colour) phenotypes, i.e. 'morphs' [26,28]. In the wild, territory owners bias their aggression towards opponents that are of the same colour morph as themselves [19]. Given that different colour morphs share the same habitats, such a pattern of aggression is expected to benefit whichever colour morph has the lowest frequency, helping to explain how a novel morph can establish in a population and then coexist with other morphs [18,21]. Furthermore, in the laboratory, gold individuals socially dominate similar-sized, dark coloured fish [28]. Nevertheless, in polymorphic populations, typically only 10% or less of the adult individuals are of the gold morph, with the rest being dark [26,29]. Thus, additional selection pressures are probably at play in countering the frequency-dependent advantage of the gold morph. In this regard, it has been suggested that a higher predation risk may select against gold individuals [28,30,31] but the evidence has so far been mixed [32–34] and, hence, it is unlikely that differences in predation (if any) are solely responsible for the low frequency of gold morph individuals in natural populations.

In the current field-based study, we considered another possibility: interspecific aggression. Specifically, we experimentally investigated territorial aggression, as displayed by a key heterospecific territorial competitor, towards dark and gold individuals in colour polymorphic *Amphilophus* cichlid fish. In particular, we assessed the possibility that heterospecific aggression is biased in a way that has the potential to contribute to colour morph frequencies encountered in the wild.

2. Material and methods

(a) The study system

This field-based study was conducted in Lake Xiloá, Nicaragua (latitude 12° 12.8′ N; longitude 86° 19.0′ W) over the course of two breeding seasons (December 2010–January 2011 and December 2013–January 2014). The lake supports more than 10 cichlid species

Figure 1. Attacking male Nicaragua cichlid, *Hypsophrys nicaraguensis*, Lake Xiloá, Nicaragua.

whose breeding seasons are largely overlapping [35–37]. Typically, irrespective of species, when pairs are ready to spawn, they claim a territory on the lake floor, and then aggressively defend the territory until the juveniles are ready to disperse [35,38]. This aggressive behaviour is directed against both conspecific and heterospecific individuals [35,37], with the patterns of niche overlap and commensal interactions potentially fine-tuning the intensity of aggression [35,38,39]. Thus, territorial aggression is directed towards competitors for territory space (both conspecific and heterospecific), brood predators (both conspecific and heterospecific), as well as conspecific sexual competitors [35,37,40].

Here, we focused on patterns of heterospecific aggression directed towards colour polymorphic *Amphilophus* species that share their breeding grounds with other, concurrently breeding cichlid species. One such species is the Nicaragua cichlid, *Hypsophrys nicaraguensis* (also known as the butterfly cichlid, parrot cichlid and moga; figure 1). Anecdotal evidence suggests that *H. nicaraguensis* parents are very effective at excluding many species from close proximity to their territories [38]. Previous work also suggests that *H. nicaraguensis* breeding pairs are often neighbours with *Amphilophus sagittae* and, to a lesser extent, *Amphilophus xiloaensis* ([19,35], T.K.L. 2010-2014, personal observations). Both of these *Amphilophus* species exhibit the distinct gold and dark colour morphs, with the frequency of the gold morph being below 10% and close to 20% in *A. sagittae* and *A. xiloaensis*, respectively [29].

Focusing on the interactions between *H. nicaraguensis* and the colour polymorphic *Amphilophus* species with which it co-occurs, our study comprised two distinct components. First, we carried out underwater field surveys to quantify the territorial neighbours of breeding pairs of both *H. nicaraguensis* and *A. sagittae*, the former being putatively the most common colour polymorphic neighbour of the latter (see the electronic supplementary material for detailed field survey methods). The aim here was to confirm that these taxa do, indeed, share overlapping territorial distributions. Second, we carried out a field experiment to investigate the aggressive responses of *H. nicaraguensis* when presented with dummy models of sympatric cichlid species, including models of gold and dark coloured *A. sagittae* and *A. xiloaensis*. This was done to test whether, in fact, heterospecific aggression is biased towards individuals of a particular colour morph—a pattern of aggression that could help to elucidate the observed colour morph frequencies in the wild. The methods of this experiment are detailed below.

(b) Aggression biases by *Hypsophrys nicaraguensis*

We experimentally assessed heterospecific aggression by the focal territorial species, the Nicaragua cichlid, *H. nicaraguensis*.

A scuba diver sequentially presented *H. nicaraguensis* pairs with four different stimulus types (with a haphazard order of presentation for each *H. nicaraguensis* pair; see below): (i) *Amphilophus* (*A. sagittae* and *A. xiloaensis*) individuals of the dark morph in breeding coloration (mostly solid dark); (ii) *Amphilophus* individuals of the dark morph in non-breeding coloration (grey with dark vertical markings); (iii) *Amphilophus* individuals of the gold morph (which, in contrast with the dark morph, looks the same irrespective of whether or not it is breeding) and (iv) individuals of the jaguar cichlid, *Parachromis managuensis*, from Lake Xiloá in adult coloration. The latter is a species whose background coloration can range from yellowish to beige, and was chosen as a control to allow us to disentangle between a more general aggressive response towards light-coloured intruders and a specific aggression toward the lighter (i.e. gold) morph of the *Amphilophus* species.

We controlled for the stimulus phenotype and behaviour by using model intruders that were all the same size (16 cm in length). Hand-made models have been successfully used as stimuli to elicit ecologically relevant behaviours in a range of fish species (reviewed in [41]), including field-based studies of territorial aggression in cichlids [19,42,43]. The use of models (or 'dummies') have a significant advantage over the use of live stimulus animals, by allowing us to explicitly control for confounding factors that might otherwise arise from differences in the behaviour of the stimulus animals. However, instead of using the more traditional wax or painted shapes employed by other researchers [41,44], we chose to use more realistic-looking models based on photographs of wild-caught fish following the methods of Lehtonen [19]. Specifically, the intruder models were made by gluing waterproof photographic colour prints of a photo of a lateral side of a live or freshly euthanized fish of the desired type onto both lateral sides of a fish-shaped floating plate (thickness = 6 mm). The model was then attached to a sinker with a thin, transparent fishing line, allowing it to float in a natural position approximately 15 cm above the lake bottom. The numbers of models prepared this way were as follows for the four types of stimuli: (1) *Amphilophus* dark in breeding coloration: $n = 11$, (2) *Amphilophus* dark, non-breeding coloration: $n = 11$, (3) *Amphilophus* gold (looks the same independent of the breeding phase): $n = 12$, and (4) *P. managuensis*: $n = 10$. For each of the stimulus types involving *Amphilophus* (types 1–3), we had photographed both of the colour polymorphic Lake Xiloá species, *A. sagittae* and *A. xiloaensis*. In half of the replicates, we used *A. sagittae* models exclusively (for the stimuli types 1–3), and *A. xiloaensis* models were used for the rest of the replicates. Including both *A. sagittae* and *A. xiloaensis* models provided us with the opportunity to test whether any differences in aggression directed by *H. nicaraguensis* towards gold and dark morphs is species specific or a more generalized response towards the different colour morphs. The *P. managuensis* models (stimulus type 4) were prepared using photographs of adult specimens from Lake Xiloá. Each model ($n = 44$) was prepared using a photograph from a different fish. A unique combination of the four different model types was used in each of our 24 replicates (see below).

Each replicate was initiated by placing a dummy at a distance approximately 40 cm from the centre of the *H. nicaraguensis* territory. We then counted the total number of aggressive encounters by territory-owners (both male and female) towards the dummy for 5 min, giving the total 'aggression rate' [19]. After a 5 min resting period (with all models out of sight), we repeated the procedure with each of the remaining model types, one after the other, so that each pair of territorial *H. nicaraguensis* was exposed sequentially to all four types of models. The 5-min resting period was chosen for two main reasons. First, we wanted to minimize any temporal changes in both abiotic (e.g. lighting) and biotic (e.g. other fish moving in and out of the area) conditions. Second, we considered a 5-min interval to be ecologically relevant as territory holders commonly encounter a range of different

species within a span of a few minutes ([37], personal observations). We sampled 24 biparentally defended *H. nicaraguensis* territories, with every possible order of presenting the four different model types ($n = 24$ different combinations) used only once to control for any potential order effects.

To assess the effects of the type of the model intruder (1–4), the species of *Amphilophus* used for the models (*A. sagittae* versus *A. xiloaensis*), the sex of the focal territory holders (male versus female) and interactions between these effects, we analysed the aggression data using a generalized mixed model with a negative binomial error distribution, as appropriate for overdispersed count data [45]. To account for non-independence of the actions of a male and female defending a territory, territory ID was added as a random effect. We simplified the model in a stepwise fashion by assessing whether we could refit the model without the least significant term of the highest remaining order. We applied χ^2-tests (with $p = 0.05$ as the cut-off point) for this purpose. We used R v. 3.1.0 software (R Development Core Team) for the analyses.

3. Results

(a) Field survey: distribution of territorial neighbours

For the focal *H. nicaraguensis* territories ($n = 113$), the most common nearest neighbours were conspecifics (34%, or $n = 38$ territories). In turn, 25% of the territories ($n = 28$) had a pair of *A. sagittae* as the closest neighbour, of which 26, 0 and 2 were dark × dark, gold × gold and mixed colour pairs, respectively (electronic supplementary material, table S1).

Focusing on *A. sagittae* territories ($n = 200$), the most common neighbours were *H. nicaraguensis* ($n = 104$ territories, or 52%), followed by conspecifics ($n = 43$; 21.5%). Regarding the colour morphs of these focal territory holders, the proportion of *H. nicaraguensis* neighbours was 44.5% (45/101), 50% (21/42) and 66.5% (38/57) for dark × dark, gold × gold and mixed pairs, respectively (electronic supplementary material, table S1). This indicates no significant difference between the different *A. sagittae* pair types in how often they had *H. nicaraguensis* as the closest neighbour (G-test of independence with Williams' correction, $G = 2.142$, d.f. = 2, $p = 0.34$). Hence, in line with our prediction, *H. nicaraguensis* was a very frequent territorial neighbour of both colour morphs of *A. sagittae*. See the electronic supplementary material, table S1 for the complete list by species of the closest territorial neighbours.

(b) Aggression biases by *Hypsophrys nicaraguensis*

When we applied a generalized mixed model to assess the rate of aggression (in relation to the territory-holder sex, intruder model type and whether *Amphilophus* intruder models were *A. sagittae* or *A. xiloaensis*), we found all interactions to be non-significant (in all cases $p > 0.10$). Similarly, there was no significant difference in aggressive responses of *H. nicaraguensis* territory holders towards *A. sagittae* and *A. xiloaensis* intruder models ($\chi^2 = 0.01$, d.f. = 1, $p = 0.92$). A model refitted with the remaining effects, i.e. territory-holder sex and type of the model intruder, indicated that there was no significant difference in aggression directed to the breeder versus non-breeder models of the dark morph ($z = 1.16$, $p = 0.25$). We, therefore, fitted a new model in which the two were combined ($\chi^2 = 1.34$, d.f. = 1, $p = 0.25$). This final model showed that males were more aggressive than females ($z = 3.80$, $p = 0.0001$; figure 2)
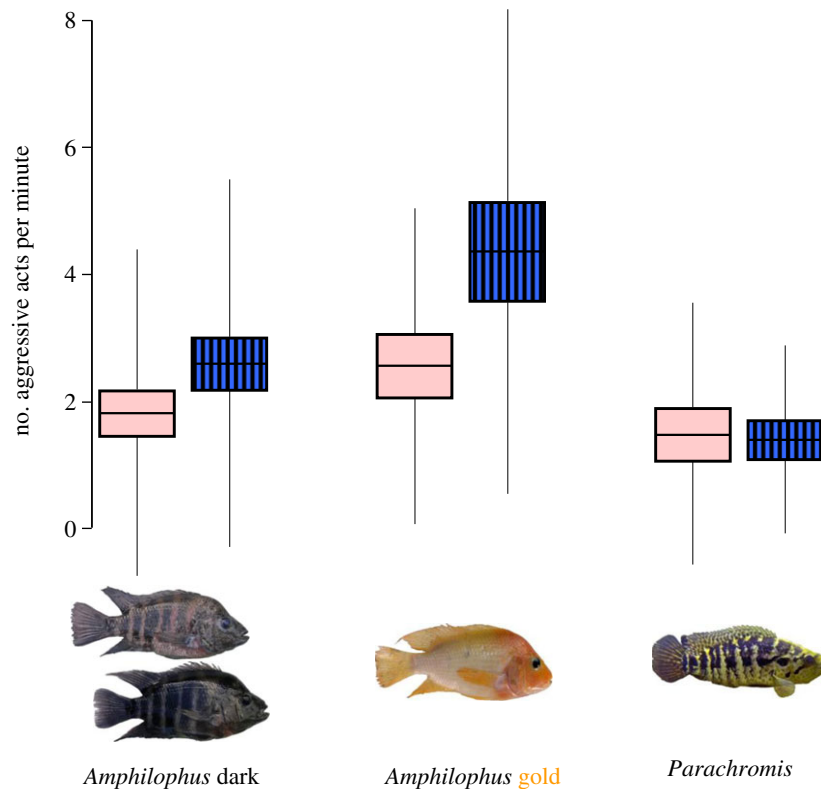
3

Figure 2. The total rate of aggression towards model opponents by female (pink boxes) and male (blue boxes with vertical stripes) *Hypsophrys nicaraguensis*. The different types of model intruders are pictured at the bottom of the graph. Central horizontal lines within the boxes, margins of the boxes and whiskers indicate means, s.e. and s.d., respectively. The number of *H. nicaraguensis* territories sampled, and hence the sample size for each box, was 24.

and aggression was significantly higher towards the gold than dark morph models ($z = 3.03$, $p = 0.0024$; figure 2). In turn, compared with the control (*Parachromis*) models, aggression rate towards dark morph models (breeders and non-breeders combined) tended to be higher (figure 2) but not significantly so ($z = 1.80$, $p = 0.072$).

4. Discussion

We found that *H. nicaraguensis* is a key territorial neighbour of *A. sagittae* in Lake Xiloá. This result suggests a high potential for territorial interactions between the two species, which is supported by our underwater observations during this and earlier studies [19,38]. Indeed, as competition within and between species for territory space can be intense in multi-species breeding grounds, such as those occupied by many *Amphilophus* species [35,46,47], a high occurrence of these interspecific disputes is likely to have a significant effect on the territory-holders' success in territorial acquisition and maintenance. In this respect, while interactions with conspecific competitors probably have the greatest impact on territory acquisition in *A. sagittae*, our results suggest that *H. nicaraguensis* is the most important of the heterospecific competitors. In particular, approximately half of *A. sagittae* territories, independent of the colour morph of the territory holders, had a pair of *H. nicaraguensis* as their closest neighbour. Interestingly, close proximity of a *H. nicaraguensis* pair has been suggested to benefit small cichlids, possibly due to *H. nicaraguensis* being aggressive towards many larger species [38]. In this respect, the high level of aggression that *H. nicaraguensis* directs towards the gold-coloured fish (see below) may contribute to the complete absence of gold × gold *A. sagittae* pairs as the nearest neighbours of

the focal *H. nicaraguensis* territory holders. However, this pattern of neighbouring territories could similarly be explained by the low frequency of the *A. sagittae* gold morph [29], especially given that when we focused on *A. sagittae* territories, *H. nicaraguensis* was found to be the most common neighbouring species independent of the colour morph(s) of the territory holders. Because of the deeper water depth preferred by breeding *A. xiloaensis* and their lower abundance at the study site ([29], personal observations), neither colour morph of this species was among the nearest neighbours in the current assessment of territorial interactions. Our observations nevertheless suggest that at the sites where *H. nicaraguensis* and *A. xiloaensis* are neighbours, they may similarly compete for territory space and potentially other resources. Indeed, the results of our model presentation experiment indicated that *H. nicaraguensis* reacted similarly towards *A. sagittae* and *A. xiloaensis* models. The results also remain qualitatively the same even if *A. xiloaensis* models are excluded.

In our intruder model presentation experiment, *H. nicaraguensis* directed more aggression towards gold than dark morph *Amphilophus* models, regardless of whether the latter were in breeding or non-breeding coloration. This result suggests that gold-coloured *A. sagittae* trying to establish a territory are likely to experience more aggression than dark *A. sagittae* by their key heterospecific competitors, *H. nicaraguensis*. Being subject to a higher rate of aggression should be costly to the gold morph. For example, the proportion of gold individuals—relative to dark ones—that are able to establish, and successfully maintain, an adequate territory may be lower than it would be in the absence of the biased heterospecific aggression. Interestingly, such a disadvantage could help to explain the morph distributions observed in the wild, e.g. the lower than expected frequency

of individuals of the gold compared with dark morph in *A. sagittae* [19,29]. Furthermore, the different breeding habitat preferences of *A. xiloaensis* [29] may allow that species to have less contact with many other cichlid species, such as *H. nicaraguensis*, potentially explaining why it has a higher frequency of gold individuals than *A. sagittae* [29]. Indeed, if such biases in heterospecific aggression are also displayed by other species besides *H. nicaraguensis*, heterospecific aggression could help to explain the low frequencies of the gold morph more generally, especially within the Midas cichlid complex. Our results, therefore, suggest that heterospecific aggression should be taken into account when the complex interactions that are involved in maintenance of a stable (colour) polymorphism [2–4,48] are considered.

Why should *H. nicaraguensis* territory holders be more aggressive towards gold than dark *A. sagittae*? One possible explanation is that the gold morph individuals of *A. sagittae* appear more similar in colour to *H. nicaraguensis* than dark individuals (figures 1 and 2). In particular, phenotypically more similar species and individuals are known to be treated more aggressively than species (or individuals) that are less alike with the aggressor [20,49–52], as has been shown, for example, in the conspecific context, in Lake Xiloá for *A. sagittae* [19]. Such a pattern of aggression is usually assumed to stem from territory holders recognizing intruders similar to themselves as more serious competitors [18,24]. Currently, we do not have data for assessing whether the two colour morphs differ in terms of their status as competitors or threat to *H. nicaraguensis*. Such a threat difference is, however, feasible because motivational states of dark individuals may be more evident than those of gold individuals, because of the latter having a lower capacity to signal through adjustment of colour patterns [28,31]. In this respect, one prediction of the 'uncertainty hypothesis' (*sensu* Peiman & Robinson [22]) is that individuals should be more wary towards intruders whose intentions are uncertain. According to this hypothesis, *H. nicaraguensis* should be particularly alert when interacting with the gold morph.

It is also possible that the bright coloration of the gold morph *per se* might have resulted in a higher level of aggression directed towards them, with orange and reddish coloration being often associated with aggressive signalling and interactions in a wide range of taxa. In particular, not only are individuals and morphs with such colours commonly dominant in interactions among conspecific individuals, as in the Gouldian finch ([53] and references therein), but brightly coloured individuals may also be challenged more aggressively, as has been shown in reptiles [54] and fish [55,56], including cichlids [57]. Indeed, although niche overlap has been offered as one of the main predictors of the level of heterospecific aggression [22], it does not always explain its intensity, as in lacertid lizards [58]. In cichlids, it also remains possible that because the dark individuals are—and historically have been—much more numerous [26,29,59], *H. nicaraguensis* territory holders may be more familiar with this intruder type and, because of learning effects such as the 'dear enemy effect' and stimulus habituation [60,61], react more intensively to less familiar gold individuals. Furthermore, because of the considerably lower background matching ability of the gold morph [31], individuals of the gold morph are likely to be more conspicuous to *H. nicaraguensis* territory holders.

The possibility that *H. nicaraguensis* territory holders were simply reacting to phenotypic similarity between themselves and the intruders might predict that they should also be more aggressive towards the *P. managuensis* models that—similarly to them—have a paler (yellowish and sometimes beige) coloration (figure 2). This, however, was not the case: if anything, *H. nicaraguensis* territory holders directed less aggression toward *Parachromis* than any other model type. One explanation for low rates of aggression between the species is that they seem to venture into each other's territories much less often than *H. nicaraguensis* and *A. sagittae* do (personal observations): in the current study none of the assessed *H. nicaraguensis* territories had *Parachromis* territories as their close neighbours. More generally, compared with the *Amphilophus* species, the potentially smaller niche overlap between *Parachromis* and *H. nicaraguensis* could explain why *H. nicaraguensis* might direct a reduced level of aggression towards the former; heterospecific aggression is generally predicted to have a positive association with the magnitude of niche overlap [22]. We also note that although small *Parachromis* in their typical dark juvenile coloration often predate upon fry of other cichlids ([37], personal observations), we have never observed larger *Parachromis* individuals in the paler adult coloration to do so, despite having conducted extensive fieldwork in the area (e.g. [19,29,38,47]), suggesting that they might not pose a direct threat to cichlid parents. This could be relevant as all of the *P. managuensis* models used in our study displayed adult coloration. Intriguingly, *H. nicaraguensis* sometimes engage in a peculiar species interaction with *Parachromis dovii*, in which a male of the former appears to help the latter in territory defence [39]. We cannot exclude the possibility that this species interaction could also contribute to the lower observed rates of aggression between *H. nicaraguensis* and *P. managuensis*, with the latter being quite similar in appearance to *P. dovii* (personal observations).

To conclude, the results of this study show that *H. nicaraguensis* is capable of a sophisticated assessment of territorial intruders based on their phenotype alone when controlling for any behavioural differences (by using model intruders). In particular, they were more aggressive towards the gold than dark morph of *A. sagittae* and *A. xiloaensis*, while tending to show less aggression towards yellowish/pale *Parachromis* models. Importantly, the higher rate of aggression directed towards gold *Amphilophus* individuals is likely be an important cost that could help to explain their lower than expected frequency. More broadly, greater attention should be given to the role of heterospecific aggression in explaining observed patterns of morph frequencies and coexistence.

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